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PRINCIPAL INVESTIGATOR: Lynn C. Hartmann, M.D.

CONTRACTING ORGANIZATION: Mayo Clinic, Rochester  
Rochester, MN 55905

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14. ABSTRACT  Optimal early detection and prevention strategies for breast cancer are predicated on our ability to identify individuals at significantly increased risk for this disease. The purpose of this Center is to bring molecular risk prediction for breast cancer into the clinical area. This will require progress on three fronts of scientific endeavor: (i) Establishment of a tissue repository of benign breast disease; (ii) Assessment of potential biomarkers of risk in this tissue set and (iii) Discovery of new, potentially relevant biomarkers of risk. We have made significant progress on these aims. Our current cohort comprises 9,376 women, 758 (8%) of whom have been diagnosed with breast cancer since the time of their benign biopsy. We established our tissue repository of benign breast tissue and have collected the subsequent breast cancer tissue. We assessed the significance of benign histology in predicting risk of future breast cancer, examining in detail the role of proliferative disease, atypia, papillomas, radial scars and involution. We explored the link between centrosome amplification, COX-2 expression and breast cancer outcomes and are currently exploring the significance of p16, ER and MIB-1. We have begun our work with Wayne State to characterize the histopathology in a cohort of African American women. Our focus in 2007-2008 will be on the Wayne State cohort and exploring additional molecular markers.					
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## INTRODUCTION

Our fifth year Center of Excellence report details a total of 52 months of work involving human subjects. Delays during initial approval processes led to some delay in the start-up funding for the human subjects portion of the grant.

Three main aims of scientific activity exist within our Center of Excellence: 1) the establishment of a large tissue repository from a retrospective cohort of women with benign breast disease (BBD) (1967-1991); 2) the application of potential biomarkers of risk to this archival tissue set; and 3) the discovery of new, potentially relevant biomarkers of risk in fresh and frozen specimens of BBD. The Center includes a multi-institutional team of basic scientists, pathologists, epidemiologists, clinicians, statisticians, and advocates (Mayo Clinic; University of California San Francisco (USCF); Wayne State).

### Task 1: Establish Retrospective Cohort of BBD and Nested Case-Control Study

#### A. Complete cohort follow-up

We reported the details in our 2006 report. This task has been completed.

#### B. Validate reported breast cancers

We reported the details in our 2006 report. This task has been completed.

#### C. Match appropriate controls to known breast cancer cases

We described this process in our 2004 report. This task has been completed.

#### D. Construct test set for preliminary evaluation of markers

We described the construction of our test set in our 2004 report. This subset consists of 124 cases and their two closest controls selected from the entire study period.

#### E. Construct validation set from remaining breast cancer cases, each matched with two controls.

The remaining cases and controls will serve as the validation set.

### Task 2: Biomarkers in Archived Tissues from Cases and Controls

#### A. Retrieve tissue slides/blocks of BBD specimens for all cases and controls

We reported details in our 2006 report. This task has been completed.

#### B. Characterize benign histopathology

##### 1. General findings

In 2006 we reported the benign histology for our entire cohort. This objective has been completed. We published the general histology findings in July 2005 in the *New England Journal of Medicine*.

##### 2. Atypia

We reported on our atypia results in our 2006 report. These results were just published in the the July 1, 2007 issue of *Journal of Clinical Oncology* (Appendix A). The following highlights the major findings:

Although atypical hyperplasia is a well-established risk factor for subsequent breast cancer, data regarding long-term absolute risk and factors for risk stratification are needed. Estimates of absolute risk with long-term follow-up are not well established. We found the following:

- With a mean follow-up of 13.7 years, 66 breast cancers (19.9%) occurred among 331 women with atypia. The relative risk of breast cancer with atypia was 3.88 (95% CI 3.00-4.94).
  - Marked elevations in risk were seen with multifocal atypia (e.g., three or more foci with calcifications ((relative risk - 10.35, 95% CI, 6.13 – 16.4)).
  - Multiple foci of atypia and the presence of histologic calcifications may indicate “very high risk” status, exceeding 50% risk at 20 years.
  - Relative risk was higher for younger women, under 45 years of age (Relative risk 6.76, 95% CI 3.24-12.4)
  - Risk was similar for atypical ductal and atypical lobular hyperplasia.
  - Breast cancer risk remained elevated over 20 years, and the cumulative incidence approached 35% at 30 years.
  - A positive family history does not further increase risk in women with atypia.
- Degnim AC, Visscher DW, Berman HK, Frost MH, Sellers TA, Vierkant RA, Maloney SD, Pankratz VS, deGroen PC, Lingle WL, Ghosh K, Penheiter L, Tlsty T, Melton LJ, Reynolds CA, Hartmann LC. Stratification of breast cancer risk in women with atypia: A Mayo Cohort study. *Journal of Clinical Oncology* 2007; 25(19):2671-77.

### 3. Papillomas

These data and the publication of these data were reported in 2006.

### 4. Involution

In our 2006 report we identified that the extent of lobular involution in breast tissue is an important risk indicator for the development of breast cancer. These results were recently published in the *Journal of the National Cancer Institute* in November, 2006 (see appendix B). We found the following:

- Risk of breast cancer was associated with the extent of involution. Comparisons in relation to the Iowa SEER population revealed that the relative risk for women with no involution was 1.88 (95% CI = 1.59-2.21), partial involution 1.47 (CI = 1.33 – 1.61) and for complete involution the relative risk was 0.91 (CI = 0.75-1.10).
- Increased involution was positively associated with increased age and inversely associated with parity.
- The significant reduction in breast cancer risk noted with involution also existed in women at “high risk” based on atypia or young age

We are pleased with the accompanying editorial (see appendix C) in which the authors Henson DW, Tarone RE, Nsouli H from George Washington University Cancer Institute asserted:

- “It truly is a remarkable event when traditional pathologic observations lead to new ideas about the prevention of cancer.”
- “It is the first study.....to substantiate a hypothesis.....that delayed involution is a major risk factor for breast cancer.....”
- “One of the most striking findings in the study.....is the degree to which the strong association between extent of involution and breast cancer risk was independent of all known breast cancer risk factors that were investigated.”
- “Results of the Mayo study provide a new paradigm for breast cancer research and prevention.”

Milanese TR, Hartmann LC, Sellers TA, Frost MH, Vierkant RA, Maloney SD, Pankratz VS, Degnim AC, Vachon CM, Reynolds CA, Thompson RA, Melton LJ, Goode EL, Visscher DW. Age-related

lobular involution and risk of breast cancer. *Journal of the National Cancer Institute* 2006 98(22):1600-07.

Henson DE, Tarone RE, Nsouli H. Lobular involution: the physiological prevention of breast cancer. *Journal of the National Cancer Institute* 2006 98(22):1589-1590.

## 5. Radial Scars

The significance of radial scars to subsequent risk of breast cancer has been debated. Radial scars (RS) are benign breast lesions of uncertain etiology. The growth pattern in RS can resemble breast cancer and on mammogram a RS can be difficult to distinguish from breast cancer, prompting a biopsy. The literature is mixed about the risk of developing breast cancer following the diagnosis of a RS, leading to our interest in examining the significance of RS in the subsequent development of breast cancer. We found no increased breast cancer risk for women with radial scars when compared to the risk already present due to proliferative disease with or without atypia. Breast cancer risk was also not affected by the size or number of RS lesions.

This manuscript has been published online: Berg JC, Visscher DW, Vierkant RA, Pankratz VS, Maloney SD, Lewis JT, Frost MH, Ghosh K, Degnim AC, Brandt KR, Vachon CM, Reynolds CR, Hartmann LC. Breast cancer risk in women with radial scars in benign breast biopsies. *Breast Cancer Research and Treatment*. Published online May 22, 2007

## C. Prepare tissue slides for biomarker analyses

Tissue slides have been prepared for the test set and two other subgroups of interest: women whose breast cancer occurred within 5 years of their diagnosis (n = 174) and women whose histopathology revealed atypia (n = 336).

## D. Perform IHC of molecular markers

Our focus continues to be on the earliest possible changes that we might detect in these "pre-malignant" lesions. There is certainly no consensus on this point. Our decision was to begin with COX-2, ER alpha, MIB-1, gamma tubulin and cyclin-D, and the test set and atypia subgroup have been stained for these markers.

### 1. COX-2 in atypia

We reported these findings in our 2006 report. A manuscript has been prepared and is being circulated among the coauthors. The following highlights the points that will be made:

- COX-2 is an enzyme responsible for the elaboration of multiple bioactive mediators important in carcinogenesis. It has been shown to be overexpressed in DCIS and invasive breast cancer. We sought to evaluate its expression in atypical hyperplasia. Our hypothesis is that the expression of COX-2 will be increased in subjects with atypical hyperplasia (AH), and will be associated with subsequent breast cancer development. The pharmaceutical availability of COX-2 inhibitors makes this a particularly interesting marker to explore due to the possibility of subsequent trials to examine the effectiveness of COX-2 inhibitors in women at high-risk for the development of breast cancer.
- The intensity of immunostaining was associated with the type of AH ( $p < 0.001$ ).
- Most atypical ductal hyperplasia (ADH) (77%) had no or weak ( $\leq 1$ ) COX-2 staining
- Most atypical lobular hyperplasia (ALH) stain intensity was 2+ or 3+ positive (61.4%)
- Strong immunostaining was more likely with increasing age ( $p < 0.01$ )
  - Of the women who were <45 years at the time of biopsy, 20% had 2+ or 3+ staining
  - Of the women >55 years, 52.3% had showed 3+ staining, 33.8% had lesions that were 2+
- COX-2 intensity was correlated with the increasing number of AH foci ( $p = 0.02$ )

- Among the 39 subjects who had three or more foci, 61.6% had strong (2+ or 3+) staining
- Of the subjects who had a single AH focus (131 total) , 35.9% had strong staining
- The relative risks for subsequent breast cancer compared to a control population from Iowa SEER data, were 2.63 for  $\leq 1$  stain intensity, 3.56 for 2+ and 5.66 for 3+ (see Table below)

Table 1. Association of COX-2 staining intensity with risk of breast cancer after the diagnosis of atypical hyperplasia.

Characteristic	No. of women	No. of person-years	No. of Observed Events	No. of Expected Events <sup>1</sup>	Relative Risk (95% CI) <sup>2</sup>
Overall	235	3265	41	12.4	3.31 (2.38-4.49)
COX-2 Staining Intensity					
0-1+	130	1869	18	6.9	2.63 (1.56-4.15)
2+	71	1004	14	3.9	3.56 (1.94-5.97)
3+	34	391	9	1.6	5.66 (2.59-10.75)

1: number of events expected on the basis of Iowa Surveillance, Epidemiology and End Results (SEER) data.

2: all analyses account for the effects of age and calendar period. CI denotes confidence interval.

## 2. ER

The estrogen receptor is essential to mediate the growth regulatory signals of estrogen in normal breast tissue and serves as a therapeutic target and predictive factor in breast cancer. The extent of ER staining in a well-characterized cohort of women with atypia, to our knowledge, has not yet been reported.

We are using the Automated Cellular Imaging System III (ACIS) to evaluate the intensity and percent ER staining in 231 women with atypia. This system is able to provide automated quantification of biomarkers. The areas of atypia were identified by the study pathologist and read into ACIS with reports produced for each identified area of interest. The preliminary analysis of the 10 most intensely stained areas shows:

- A mean of 56 percent stained cells (standard deviation 30.78, range 0.00 – 99.99)
- The mean intensity was 114 (standard deviation 28.97, range 0-206 (possible range 0 -256)).
- A linear multivariate mixed model examined percent staining and intensity differences based on atypia type (lobular, ductal), cancer status, and year of biopsy after controlling for repeated measures within a woman.
  - The only difference based on this initial analysis was stronger intensity of staining and greater percentage of staining of atypical ductal hyperplasia compared to atypical lobular hyperplasia (intensity: ADH mean of 117.68, standard error 2.5224 and ALH mean of 100.32, standard error 2.4537,  $p < 0.0001$ ; percentage: ADH mean of 64.1887, standard error 2.7860 and ALH mean of 44.2121, standard error 2.6720,  $p < 0.0001$ ).

We are currently completing the ER assessments for all areas of atypia and will finalize analyses subsequently.

### 3. Proliferation Status

Upon completion of the ER readings, we are set up to use the ACIS for measuring MIB-1 (Ki-67) stained areas. All the slides have been stained for Ki-67 and are ready for analysis.

### 4. p16

Dr. Tlsty from UCSF put forward p16 as the most significant marker to take forward into testing. We have stained the atypia slides for p16 and they have been read. Analysis will be forthcoming.

### E. Perform centromere studies.

These data were presented in our 2006 report.

## **Task 3: Discovery - *In Vitro* Culturing and Gene Profiling Studies**

### A. Identify appropriate frozen proliferative BBD specimens at Mayo and Wayne State for profiling.

The purpose of these studies is to identify new, potentially relevant biomarkers in benign breast disease, markers that might correlate with subsequent breast cancer risk. When our grant was submitted, the technology was not available to do profiling studies in paraffin-embedded tissue (such as our BBD resource) and hence, we described doing profiling in frozen samples of BBD. A serious limitation of that approach, however, is that we do not have outcome information for our frozen repository samples, since these were accrued recently, and insufficient time has elapsed for the development of breast cancer. Fortunately, genomic profiling technology has proceeded significantly and there are now platforms available for us where microdissected, paraffin-embedded samples can be run. We are working currently to identify the quantity and quality of DNA and RNA that can be obtained from the paraffin-embedded samples.

### B. Obtain fresh BBD tissue from appropriate patients at Mayo and Wayne State for culturing in vitro at UCSF. Revised 1/07 Obtain fresh BBD tissue from appropriate patients at Mayo for culturing *in vitro* at UCSF.

Forty-four samples were sent from Mayo to UCSF. Five of these samples were lost to contamination.

Multiple efforts to implement a prospective collection of fresh tissue in African-American women at Wayne State proved to be logistically impossible to launch. Thus, we have moved to develop a retrospective study in an African-American cohort at Wayne State, modeled after the Mayo (Caucasian) cohort. Through a collaboration with Dr. Hind Nassar, a junior pathologist at Wayne State, an IRB-approved protocol has been developed, to access paraffin-embedded samples of benign breast disease (BBD) from African-American women at Wayne State from 1992-2001. This will allow us to begin to look at the problem of BBD in African-American women. Moreover, because the population there is covered through the Detroit SEER database, we will have information about cancer outcomes. See task 5.

### C. Culture BBD specimens and document their growth characteristics.

These data were reported in 2005.

### D. Compare genomic expression levels of DCIS markers in BBD tissues.

We reported on this task in 2006. Task completed.

### E. Profile BBD specimens.

We reported on this task in 2006. Task completed.

#### **Task 4: Statistical Analyses**

A. Establishment of relational database

This task is complete. The database is the foundation for tracking all tissue samples; entering clinical, pathologic, and molecular data; and analyzing results.

B. Enter epidemiologic and histopathologic data

This task is complete.

C. Enter culturing data (proportion of cells that break through proliferation barriers; slope of curve, etc.)

These data were entered as collected at UCSF.

D. Enter molecular data from culturing experiments (methylation of p16, p53 status, % proliferation versus apoptosis, etc.)

These data were entered as collected at UCSF.

E. Enter gene profiling data.

These data were entered as collected at UCSF.

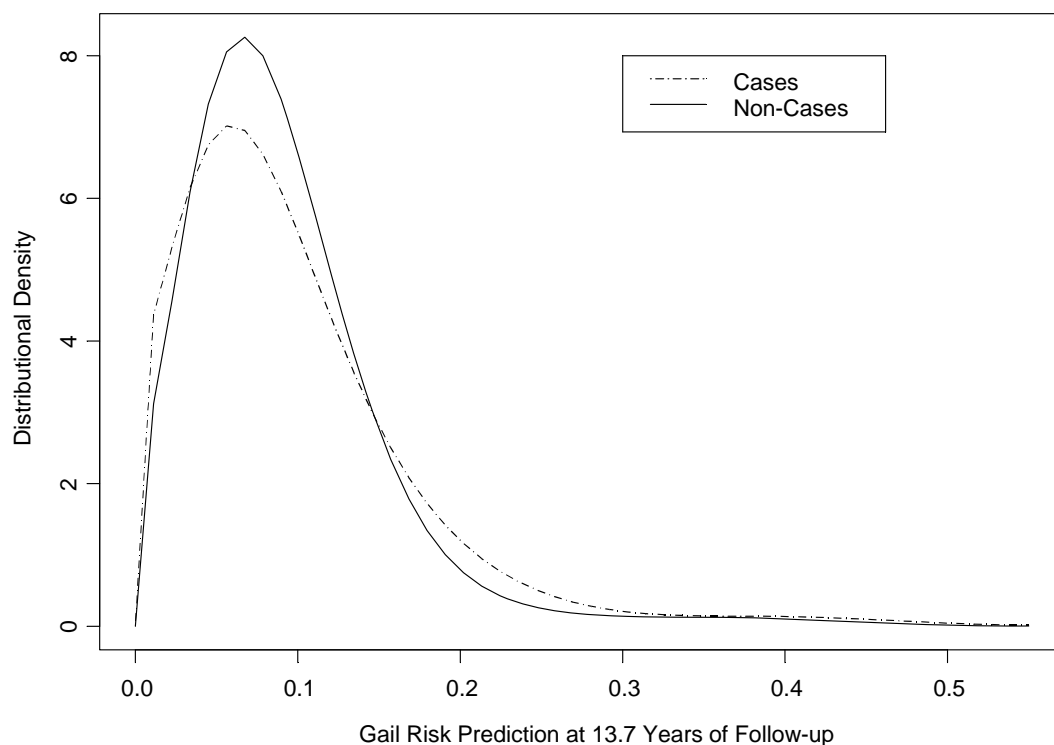
F. Calculate hazard function for breast cancer by age at BBD, family history, histology, and molecular marker data.

We have examined breast cancer risk by age at BBD, family history, histology [degree of proliferation, atypia yes/no, extent of involution, radial scar yes/no, presence of papilloma(s)], COX-2 expression, and centrosome status. We have summarized the findings in earlier sections of this report.

G. Assess accuracy of Gail model.

The Gail model is currently the main tool used in the clinical setting for risk assessment in patients with atypia. This is despite the fact that it has not been validated in this group of patients. We evaluated the Gail model in our group of women with atypia (N = 331). We used this model to predict 5 year and follow-up specific risks for each woman. The Gail model over-estimated the number of breast cancers that would occur in the first 5 years after biopsy (12.8 predicted, 8 occurred). However, we found that the Gail model underestimated the risk of breast cancer in women with atypia when using the current follow-up age of our participants (Gail model predicted 31.7 breast cancer while 58 occurred). Additionally, we found the concordance between Gail model individual-specific predicted outcomes and observed outcomes to show only modest improvement over chance alone (c-statistic 0.57, 95% CI: 0.52-0.63, p=0.011). This has significant implications for clinical practice. Healthcare providers should be cautious when using the Gail model in counseling patients with atypia regarding their risk of developing breast cancer. We are in the process of submitting a manuscript to report these findings.

**Figure 1.** Distributions of Gail model risk probabilities. Plot contains estimates for individualized risk at the end of the available follow-up. As risk predictions depend on age at BBD, and length of follow-up, the risk predictions were corrected for these factors prior to comparison.



G. Analyze expression data.

This past year we have focused on COX-2, ER, p16 and MIB1(Ki-67). We have reported our current findings under Task 2.

**Task 5: Compare Breast Cancer Risk Associated with Benign Breast Disease in African-American vs. Caucasian-American Women**

A. Identify African-American women at Wayne State University who had a breast biopsy with benign results between 1992 and 2001.

One thousand one hundred forty-five women who had a benign breast biopsy during 1999-2000 were identified. To date 240 women have been identified for 1998.

B. Retrieve slides/blocks of BBD specimens.

Three hundred twenty slides have been obtained thus far.

C. Characterize benign histology of epithelium.

Our programmer developed a data entry tool with drop down option boxes for Dr. Nassar's use. Dr. Nassar worked closely with Dr. Dan Visscher to ascertain consistency in definition and reading of cohort slides using the study pathology form. Dr. Nassar has read and entered the histology for 225 of these slides.

D. Cross list with Detroit SEER database to identify subsequent breast cancers.

Dependent on A-C.

E. Data clean-up, compare age, histology, involution status, and resulting risk with Mayo

Caucasian-American cohort and determine involution status by age of patient.

To begin once A – F accomplished.

## **KEY RESEARCH ACCOMPLISHMENTS**

- We identified the degree of risk associated with the common benign epithelial entities and the extent to which age at biopsy and family history influence the risk of breast cancer in women with proliferative or atypical lesions. The highest risk was among women who had proliferative disease with atypia, especially those of younger age.
- We identified a marked increased risk of breast cancer in women with three or more foci of atypia, especially for three or more foci with calcifications. Also, risk was higher in women diagnosed with atypical hyperplasia before age 45. Among women with atypia, risk was not affected by family history.
- We identified that a single papilloma without atypia imparts an increased risk of developing a subsequent carcinoma similar to other forms of proliferative breast disease without atypia. Atypical papilloma, particularly in the setting of multiple papillomas, imparts a breast cancer risk similar to or greater than conventional atypical ductal/lobular hyperplasias.
- We identified that the extent of lobular involution in breast tissue is an important risk indicator for the development of breast cancer. Increasing degrees of involution result in a significant reduction in breast cancer risk, even in women at “high risk” based on atypia or young age.
- We found that intense COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia and represents one potential molecular target for chemoprevention strategies.
- We found no increased breast cancer risk for women with radial scars compared to the risk already present due to proliferative disease with or without atypia.
- We identified that centrosome amplification is seen more frequently in higher risk benign lesions (e.g. atypia) and is infrequently seen in non-proliferative lesions and in proliferative lesions without atypia.
- We have identified intense p16 expression as a biomarker that identifies women with a significantly greater likelihood for recurrence after lumpectomy only for DCIS. This biomarker is presently being applied to the BBD cohort.
- We found the Gail model to predict only slightly higher than chance alone the breast cancer risk of women with atypia. The model underestimated lifetime risk and current risk of our cohort of women with atypia.

## **REPORTABLE OUTCOMES**

### **Manuscripts**

- Lewis JT, Hartmann LC, Vierkant RA, Maloney SD, Frost MH, Allers TM, Visscher DW. An analysis of breast cancer risk in women with single, multiple, and atypical papilloma. Am J Surg Pathol 2006;30(6):665-72.

- Degnim AC, Visscher DW, Berman HK, Frost MH, Sellers TA, Vierkant RA, Maloney SD, Pankratz VS, deGroen PC, Lingle WL, Ghosh K, Penheiter L, Tlsty T, Melton LJ, Reynolds CA, Hartmann LC. Stratification of breast cancer risk in women with atypia: A Mayo cohort study, Journal of Clinical Oncology 2007;25(19):2671-7.
- Milanese TR, Hartmann LC, Sellers TA, Frost MH, Vierkant RA, Maloney SD, Pankratz VS, Degnim AC, Vachon CM, Reynolds CA, Thompson RA, Melton LJ, Goode EL, Visscher DW. Age-related lobular involution and risk of breast cancer, Journal of the National Cancer Institute 2006;98(22):1600-07.
- Berg JC, Visscher DW, Vierkant RA, Pankratz VS, Maloney SD, Lewis JT, Frost MH, Ghosh K, Degnim AC, Brandt KR, Vachon CM, Reynolds CR, Hartmann LC. Breast cancer risk in women with radial scars in benign breast biopsies. Breast cancer Research and Treatment. Published online May 22, 2007.

## **Presentations**

### **Poster Presentation at annual meeting of the United States and Canadian Academy of Pathology, Atlanta, GA, Feb. 11-17, 2006.**

- Milanese TR, Hartman LC, Vierkant RA, Maloney SD, Frost MH, Pankratz VS, Visscher DW. The impact of lobular involution on breast cancer risk.

### **Podium Presentation at annual meeting of the United States and Canadian Academy of Pathology, Atlanta, GA, Feb. 11-17, 2006.**

- Berg JC, Lewis JT, Maloney SD, Vierkant RA, Hartmann LC, Visscher DW. Analysis of cancer risk in women with radial scars of the breast.

### **Podium Presentation at annual meeting of American Association for Cancer Research, April 1-5, 2006 in Washington, D.C.**

- Hartmann LC, Lingle WL, Frost MH, Maloney SD, Vierkant RA, Pankratz VS, Tlsty T, Degnim AC, Visscher DW. COX-2 expression in atypia: Correlation with breast cancer risk.

### **Poster Presentation at American Association of Cancer Research, Washington DC, April 1-5, 2006.**

- Pankratz VS, Vierkant RA, Maloney SD, Frost MH, Visscher DW, Hartmann LC. Assessment of the Gail model in a cohort of women with atypical hyperplasia.

### **Poster Presentation at Joint statistical Meetings, Minneapolis, MN, August 10, 2006.**

- Pankratz VS, Vierkant SD, Maloney SD, Hartmann LC. Epidemiologic comparisons of disease incidence among populations: The person-years approach.

### **Poster Presentation at 29<sup>th</sup> Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 16, 2006.**

- Ghosh K, Hartmann LC, Maloney D, Vierkant RA, Milanese TM, Visscher DW, Pankratz VS, Vachon CM. Mammographic breast density is inversely associated with age-related involution.

### **Poster Presentation at 29<sup>th</sup> Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 16, 2006.**

- Milanese TR, Hartmann LC, Sellers TA, Frost MH, Vierkant RA, Maloney SD, Pankratz VS, Degnim AC, Vachon CM, Reynolds CA, Thompson RA, Melton LJ, Goode EL, Visscher DW. Age-related lobular involution and risk of breast cancer.

- Lingle W, Negron V, Bruzek A, Murphy L, Riehle D, Vierkant RA, Pankratz VS, Hartmann LC, Visscher DW. Centrosome amplification is greatest in benign breast lesions associated with an increase in risk of cancer.

**Poster Presentation at American Society of Clinical Oncology's Breast Cancer Symposium, San Francisco, CA, September 2007.**

- Boughey JC, Hartmann LC, Degnim AC, Vierkant RA, Ghosh K, Vachon CM, Maloney SD, Reynolds C, Pankratz VS. Assessment of the accuracy of the Gail model in women with atypical hyperplasia.

**CONCLUSIONS**

We have made significant progress on all three aims. Specifically, we have completed the cohort follow-up by questionnaires. Our pathologist has completed readings on the benign and cancer tissue for the entire cohort. We have evaluated the significance of the benign histologic categories (NP vs. PDWA vs. AH) and examined the risks associated with specific pathologic findings including atypia, papillomas, radial scars and involution. We calculated hazard functions for breast cancer by age at BBD and family history. We applied the Gail model to our study population of women with atypia and found the concordance between predicted individual risk and actual risk to be only slightly above chance. The Gail model significantly underestimated actual risk based on length of follow-up in our cohort of women with atypia. We have stained the test and atypia subgroups for several immunohistochemical markers. We have identified COX-2 as an important marker of an increased risk of breast cancer in women with atypia and are currently exploring ER, MIB-1 and p16. Additionally, we are working closely with Wayne State to characterize the histopathology and breast cancer outcomes in a cohort of African American women with benign breast disease.

## Appendix A: Atypia Paper

## Stratification of Breast Cancer Risk in Women With Atypia: A Mayo Cohort Study

Amy C. Degnim, Daniel W. Visscher, Hal K. Berman, Marlene H. Frost, Thomas A. Sellers, Robert A. Vierkant, Shaun D. Maloney, V. Shane Pankratz, Piet C. de Groen, Wilma L. Lingle, Karthik Ghosh, Lois Penheiter, Thea Tlsty, L. Joseph Melton III, Carol A. Reynolds, and Lynn C. Hartmann

From the Divisions of General Surgery, Anatomic Pathology, Medical Oncology, Biostatistics, Gastroenterology and Hepatology, Experimental Pathology, General Internal Medicine, and Epidemiology, Mayo Clinic College of Medicine, Rochester, MN; University of California, San Francisco, San Francisco, CA; and the H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL.

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Address reprint requests to Lynn C. Hartmann, MD, Mayo Clinic, 200 First St SW, Rochester, MN 55905; e-mail: hartmann.lynn@mayo.edu.

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### ABSTRACT

#### Purpose

Atypical hyperplasia is a well-recognized risk factor for breast cancer, conveying an approximately four-fold increased risk. Data regarding long-term absolute risk and factors for risk stratification are needed.

#### Patients and Methods

Women with atypical hyperplasia in the Mayo Benign Breast Disease Cohort were identified through pathology review. Subsequent breast cancers were identified via medical records and a questionnaire. Relative risks (RRs) were estimated using standardized incidence ratios, comparing the observed number of breast cancers with those expected based on Iowa Surveillance, Epidemiology, and End Results (SEER) data. Age, histologic factors, and family history were evaluated as risk modifiers. Plots of cumulative breast cancer incidence provided estimates of risk over time.

#### Results

With mean follow-up of 13.7 years, 66 breast cancers (19.9%) occurred among 331 women with atypia. RR of breast cancer with atypia was 3.88 (95% CI, 3.00 to 4.94). Marked elevations in risk were seen with multifocal atypia (eg, three or more foci with calcifications [RR, 10.35; 95% CI, 6.13 to 16.4]). RR was higher for younger women (< 45; RR, 6.76; 95% CI, 3.24 to 12.4). Risk was similar for atypical ductal and atypical lobular hyperplasia, and family history added no significant risk. Breast cancer risk remained elevated over 20 years, and the cumulative incidence approached 35% at 30 years.

#### Conclusion

Among women with atypical hyperplasia, multiple foci of atypia and the presence of histologic calcifications may indicate "very high risk" status (> 50% risk at 20 years). A positive family history does not further increase risk in women with atypia.

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### INTRODUCTION

Atypical hyperplasia is a well-established risk factor for subsequent breast cancer. Multiple studies corroborate an approximately four-fold increased risk of breast cancer in women undergoing surgical biopsy with a finding of atypia.<sup>1-7</sup> Despite good concordance on the estimated relative risk (RR) with atypia, estimates of absolute risk with long-term follow-up are not well established. Reliable breast cancer risk estimates for women with atypia are crucial for risk-benefit analysis and decision making regarding risk-reduction strategies.

The Gail model in current use predicts a dramatically increased risk for those women who have both atypia and a family history (over that of atypia alone).<sup>8</sup> Prior published literature has stated that the

risk of breast cancer abates considerably after 10 years after a diagnosis of atypia,<sup>9</sup> whereas more recent evidence indicates otherwise.<sup>10</sup> It is also unclear whether breast cancer risk is higher in cases of atypical ductal hyperplasia (ADH) versus atypical lobular hyperplasia (ALH).

Here, we present a comprehensive description of breast cancer risk in women with atypical hyperplasia, based on 331 women with atypia in the Mayo Benign Breast Disease Cohort. Our investigation addresses the effect of family history on atypia risk, the effect of time since biopsy, the influence of ductal versus lobular histology, effects of age at atypia diagnosis, and presence of calcifications on breast cancer risk. In addition, we provide absolute risk estimates over time, and we also present a new histologic feature of atypia—multifocality—that stratifies breast cancer risk among women with atypia.

## PATIENTS AND METHODS

### Study Population

Entry criteria for the study cohort have been previously described.<sup>1</sup> Briefly, this comprises an institutional review board–approved study of women ages 18 to 85 years who had a benign breast biopsy via surgical excision during 1967 to 1991. The initial cohort included 9,087 women.<sup>1</sup> With additional follow-up, data are now available for 9,376 women, 331 (3.5%) of whom had atypical hyperplasia.

### Follow-Up

Follow-up for breast cancer events (including both invasive cancer and ductal carcinoma in situ [DCIS]) and risk-factor information were obtained for all women with atypia through the Mayo medical record and a study questionnaire. Family history was classified as negative, strong, or weak. The criteria for a strong family history were at least one first-degree relative with breast cancer before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history was considered weak.<sup>1</sup>

### Histology

All available archival hematoxylin and eosin–stained sections were evaluated by our breast pathologist (D.W.V.), without knowledge of the original histologic diagnoses or patient outcomes. The number of slides reviewed per case was variable because of the retrospective nature of the study, with a mean of 3.2 (standard deviation, 3.7). Calcifications were recorded for each case when seen histologically. A diagnosis of ADH or ALH was based on the criteria of Page et al.<sup>3,11</sup> ADH was characterized by filling and distension of involved ducts by an architecturally complex proliferation of monotonous cells forming “punched out” (cribriform-like) secondary lumens or micropapillary formations. Although well-developed examples of ADH share some morphologic features with low-grade DCIS, the latter is characterized by tumefactive growth (requiring complete involvement of  $>2$  contiguous lumens) as well as greater nuclear enlargement and hyperchromatism. For each example of atypical hyperplasia, the number of separate foci was defined. Multifocal atypia required its identification in more than one terminal duct lobular unit (TDLU) as defined by clear separation from another by nonspecialized interlobular mammary stroma. All cases of multifocal atypia were agreed on by two study pathologists (D.W.V. and C.A.R.).

The primary study pathologist (D.W.V.) identified 332 cases of atypia from the entire benign breast disease cohort of 9,376. To address concerns of reproducibility in the diagnosis of atypia,<sup>12</sup> we performed a nested study of concordance, blinding another pathologist (H.B.) to the study diagnoses in a random subset of several hundred samples from the original cohort, including nonproliferative lesions, proliferative disease without atypia, and atypical hyperplasia. Of 189 atypia samples reviewed for concordance, 165 (87.3%) atypias were similarly classified by subsequent independent review. Of the remaining 24 cases with differing interpretation, 18 were then judged to have atypia by joint review (D.W.V. and H.B.), and five of six remaining cases had atypia by review of a third “tiebreaker” breast pathologist (C.A.R.). The one case in question was excluded from further analysis, leaving a total of 331 subjects for study.

### Statistical Analysis

Follow-up was defined as the number of days from benign biopsy to date of breast cancer diagnosis, death, or last contact. We estimated RRs with standardized incidence ratios (SIRs) and 95% CIs, dividing observed numbers of incident breast cancers by expected counts. We calculated expected counts by apportioning each individual’s follow-up time into 5-year age and calendar-period categories, and applying these person-years to population-based incidence rates, thereby accounting for differences in these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population, because of its proximity to the Mayo Clinic catchment area and racial similarities compared with our cohort.<sup>1</sup> We extrapolated incidence-rate data for cohort follow-up occurring outside the SEER time-frame (1973–2002), such that person-years before 1973 were applied to 1973 to 1975 incidence rates, and person-years subsequent to 2002 were applied to

2001 to 2002 incidence rates. Assuming a two-sided test of hypothesis and a type I error rate of 0.05, we would have 80% power to detect SIRs as low as 3.61 if the expected event count is 2.5, as low as 2.97 if the count is 4.2, as low as 2.08 if the count is 10.3, and as low as 1.84 if the count is 17. Note that these expected counts reflect the approximate expected numbers of events in our cohort for women with three or more foci of atypia, two foci, one focus, and all subsets combined, respectively.

Recognizing that other biologic mechanisms may modify the association of atypia and breast cancer risk, we formally assessed the potential differential effects of these mechanisms using Poisson regression analyses. This approach allowed us to estimate SIRs with the flexibility that generalized linear models provide, such as covariate adjustment and tests for trend or heterogeneity across subgroups. For all analyses, the log-transformed expected event rate for each individual was modeled as the offset term.

We displayed observed and expected event rates using cumulative incidence curves and corresponding 95% confidence limits, accounting for the effects of death as a competing risk.<sup>13</sup> Expected events were calculated for each 1-year follow-up interval in a manner similar to that used for determining SIRs. A modified Kaplan-Meier approach was used to cumulate expected incidence over these intervals. The expected curve was then smoothed using linear interpolation.

We compared the RR of ipsilateral versus contralateral breast cancer overall and across different medical characteristics using ratios of corresponding incidence rates. When calculating incidence for ipsilateral cancer, individuals with contralateral cancer were excluded at their diagnosis date, and vice versa. Women with missing laterality, or having bilateral biopsies or cancer, were excluded for both events. The RRs are equivalent to ratios of observed events, as the approach yields identical person-years for each event type. We thus used properties of the binomial distribution to obtain exact 95% CIs for these RRs.<sup>14</sup> All statistical tests were postulated a priori and were two sided, and all analyses were conducted using the SAS software system (SAS Institute, Cary, NC).

## RESULTS

### Characteristics of Patients and Pathologic Specimens

A total of 331 women with atypia were identified in our cohort between 1967 and 1991. In Table 1, we present the patients’ vital status, breast cancer status, family history, age at biopsy, year of biopsy, indication for biopsy, and histologic features. Women were likely to be older than 55 at diagnosis of atypia (55.9%), and 42.9% had a family history of breast cancer (23.5% with a strong family history). Histologic findings included calcifications in most cases of atypia (68.6%); most cases (60.1%) had only one focus of atypia. The relative percentages of women with one, two, and three or more foci of atypia remained stable over the time period of the cohort. The proportions of women with ADH and ALH were similar.

### Subsequent Breast Cancer Risk and Modifying Factors

The 331 women with atypia were followed for a total of 4,543 person-years (mean 13.7 years), with 66 (19.9%) observed breast cancer events to date. The histologic types are known in 61 of these, with 53 (86.9%) of 61 invasive cancers and eight (13.1%) of 61 DCIS. The majority of invasive cancers were ductal type (47 of 53, 89%), and the remaining six invasive lobular cancers were divided between the ALH and ADH subgroups. Table 2 shows the estimated RRs for breast cancer associated with various characteristics. The overall group with atypia demonstrates a four-fold RR of breast cancer (RR, 3.88; 95% CI, 3.00 to 4.94) compared with the general population.

### Family History

There were no significant differences in RR seen among the subgroups with a strong family history (RR, 3.59; 95% CI, 1.96 to

**Table 1.** Clinical and Histologic Characteristics Among the 331 Women With Atypical Hyperplasia From the Mayo Benign Breast Disease Cohort Study

Characteristic	No.	%
<b>Vital status</b>		
Deceased	101	30.5
Alive	230	69.5
<b>Breast cancer status</b>		
Noncase	265	80.1
Case	66	19.9
<b>Age at biopsy, years</b>		
Mean	58	
SD	12	
<b>Family history of breast cancer</b>		
Unknown	42	
None	165	57.1
Weak	56	19.4
Strong	68	23.5
<b>Age at BBD, years</b>		
< 45 at BBD Dx	46	13.9
45-55 at BBD Dx	100	30.2
> 55 at BBD Dx	185	55.9
<b>Year of BBD</b>		
1967-1971	15	4.5
1972-1976	35	10.6
1977-1981	40	12.1
1982-1986	96	29.0
1987-1991	145	43.8
<b>Indication for biopsy</b>		
Unknown	6	
Palpable mass	139	42.8
Mammographic abnormality	186	57.2
<b>Calcifications</b>		
Without calcifications	104	31.4
With calcifications	227	68.6
<b>Histologic subtype</b>		
Lobular	175	52.9
Ductal	142	42.9
Lobular and ductal	14	4.2
<b>No. of foci of atypia</b>		
1	199	60.1
2	81	24.5
≥ 3	51	15.4

Abbreviations: SD, standard deviation; BBD, benign breast disease; Dx, diagnosis.

6.03), a weak family history (RR, 5.59; 95% CI, 3.20 to 9.09), or a negative family history (RR, 3.81; 95% CI, 2.60 to 5.37; Table 2; Fig 1A).

#### Age at Biopsy

Women with atypia diagnosed at younger age had a higher RR compared with age-matched expected rates (Table 2; Fig 1C). The RR was 6.76 at age less than 45, 5.10 at age 45 to 55, and 2.87 at age greater than 55 years ( $P$  for trend = .01). The increased risk seen in younger women was not due to a positive family history, because there was no difference in risk for women with and without a family history in each age subgroup (data not shown).

#### Number of Foci of Atypia

Increasing risk was seen with increasing foci of atypia: RR = 2.33 with a single focus, 5.26 for two foci, and 7.97 for three or more foci,

with a highly significant test for trend ( $P < .001$ ; Fig 1B). The increased risk seen with multiple foci of atypia was not due to predominance of young (higher risk) women in those subgroups; women younger than 45 years constituted only 4.94% and 7.84% of the subgroups with two and three foci of atypia, compared with 19.1% of the subgroup with one focus. Multivariate Poisson regression analysis also confirmed that young age and multifocality contributed independently to increased risk.

#### Calcifications

Risk was dramatically increased in the small group of women ( $n = 38$ ) with both calcifications and three or more foci of atypia (RR, 10.4; 95% CI, 6.13 to 16.4). However, women with calcifications and less than three foci of atypia (RR, 3.1) had risk similar to that of patients with fewer than three foci of atypia and no calcifications (RR, 3.31).

#### Histologic Type of Atypia

Histologic type of atypia did not affect breast cancer risk, because the RR of breast cancer was the same for ADH and ALH, although the few individuals with both histologic types may have higher risk (Fig 1D).

#### Indication for Biopsy

Breast cancer risk was similar whether a palpable or mammographic concern prompted the biopsy.

#### At-Risk Time Interval and Cumulative Incidence of Breast Cancer

The RR of breast cancer for the entire group with atypical hyperplasia was elevated persistently beyond 15 years, with a 20-year cumulative risk of 21% (95% CI, 14% to 28%) and a 25-year cumulative risk of 29% (95% CI, 20% to 38%; Fig 2). Stratification based on number of foci of atypia demonstrates a cumulative incidence of 18% for a single focus, 45% for two foci, and 48% for three or more foci of atypia at 25 years of follow-up (Fig 3).

#### Laterality of Breast Cancer Risk

Of the 66 women with atypia who subsequently developed breast cancer, side of cancer and side of atypia are known in 57 cases. Although cancer was more frequent in the ipsilateral breast, this difference was not statistically significant for the overall group with atypia (RR, 1.38 for ipsilateral v contralateral event; 95% CI, 0.79 to 2.21). However, the 32 women with atypia who developed breast cancer within 10 years of their benign biopsy were 2.2 times more likely (95% CI, 1.02 to 4.86;  $P = .05$ ) to develop cancer in the same breast versus the opposite breast. Women with ADH had higher ipsilateral risk (RR, 1.50; 95% CI, 0.62 to 3.82), and women with three or more foci also had higher risk of ipsilateral breast cancer (RR, 2.20; 95% CI, 0.71 to 4.52), although these increases did not reach statistical significance due in part to small numbers of events and modest statistical power for these analyses. Women with ALH had similar cancer risk in both breasts (RR, 1.08; 95% CI, 0.45 to 2.14).

## DISCUSSION

Having reliable breast cancer risk estimates for women with atypical hyperplasia is imperative in order to tailor their care appropriately. For women with atypia, the Gail model is the only model available for risk

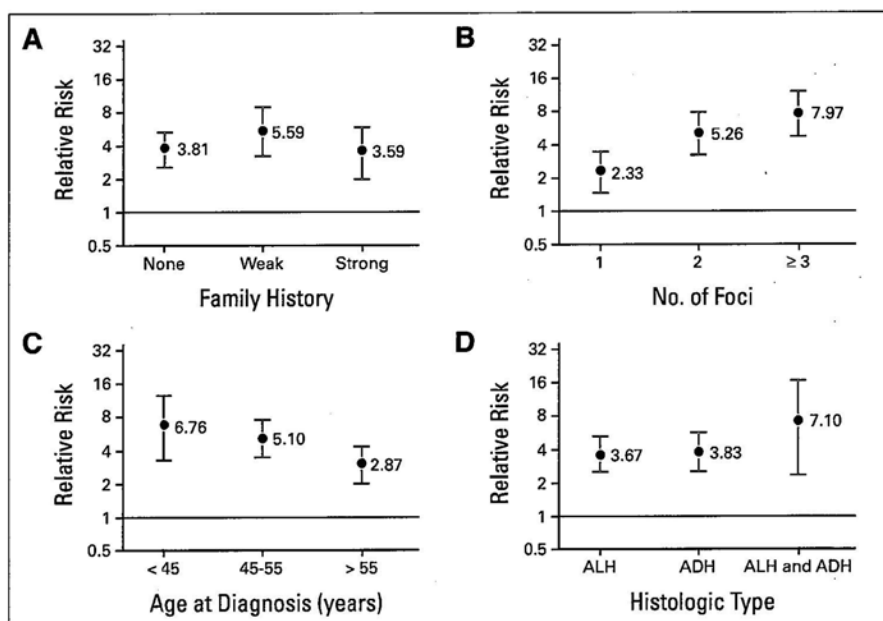
**Table 2.** Risk Factors for Breast Cancer Among the 331 Women With Atypia From the Mayo Benign Breast Disease Cohort Study

Variable	No.	Person-Years	Observed Events	Expected Events	RR	95% CI
Overall atypia group	331	4,543	66	17.0	3.88	3.00 to 4.94
Age at benign biopsy, years						
< 45	46	678	10	1.5	6.76	3.24 to 12.40
45-55	100	1,540	26	5.1	5.10	3.33 to 7.48
> 55	185	2,325	30	10.4	2.87	1.94 to 4.10
No. of foci of atypia						
1	199	2,792	24	10.3	2.33	1.49 to 3.46
2	81	1,086	22	4.2	5.26	3.29 to 7.96
≥ 3	51	665	20	2.5	7.97	4.87 to 12.30
Calcifications						
Without	104	1,529	18	5.6	3.21	1.90 to 5.08
With	227	3,013	48	11.4	4.21	3.10 to 5.58
< 3 foci	189	2,536	30	9.7	3.10	2.09 to 4.43
≥ 3 foci	38	478	18	1.7	10.4	6.13 to 16.40
Histologic subtype						
Lobular	175	2,535	34	9.3	3.67	2.54 to 5.13
Ductal	142	1,815	27	7.0	3.83	2.53 to 5.58
Lobular and ductal	14	194	5	0.7	7.10	2.31 to 16.5
Family history of breast cancer						
None	165	2,226	32	8.4	3.81	2.60 to 5.37
Weak	56	763	16	2.9	5.59	3.20 to 9.09
Strong	68	1,029	14	3.9	3.59	1.96 to 6.03
Indication for biopsy						
Palpable mass	139	2,068	33	7.2	4.55	3.13 to 6.39
Mammographic abnormality	186	2,409	32	9.5	3.36	2.30 to 4.74

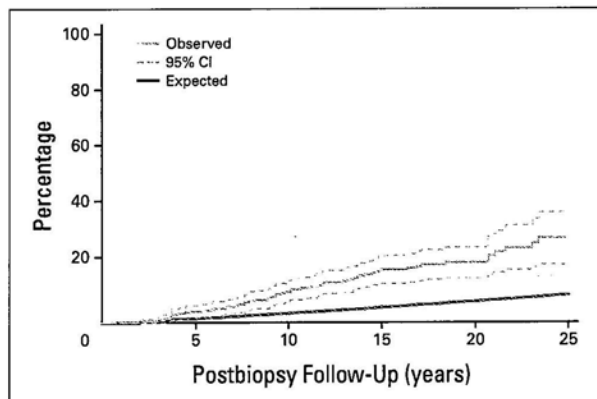
NOTE. RR and CI represent standardized incidence ratio and 95% confidence limits, comparing observed number of events to those expected based on Iowa Surveillance, Epidemiology, and End Results data. All results account for the effects of age and calendar period. Abbreviation: RR, relative risks.

prediction.<sup>8</sup> In this model, calculations of risk for women with atypia and a family history are dramatically higher, based on prior evidence from the Nashville study.<sup>2</sup> Therefore, for a 50-year-old

white woman with menarche at age 12, first birth at 24, and atypia on breast biopsy, the predicted lifetime risk of breast cancer is 17.5%. If that same woman also has a first-degree relative with



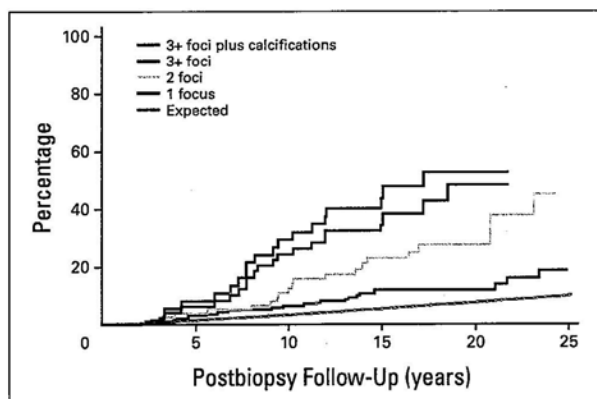
**Fig 1.** Stratification of breast cancer risk for women with atypical hyperplasia. (A) Family history, (B) number of foci, (C) age at diagnosis, and (D) histologic type. Relative risks expressed as standardized incidence ratios and 95% confidence limits, comparing observed number of events to those expected based on Iowa Surveillance, Epidemiology, and End Results data. All results account for the effects of age and calendar period. Horizontal lines at 1.0 reflects the overall expected relative risk in the cohort. ALH, atypical lobular hyperplasia; ADH, atypical ductal hyperplasia.



**Fig 2.** Cumulative risk of breast cancer over time. Observed cumulative breast cancer incidence among women with atypical hyperplasia, with 95% CIs represented by stippled lines. Expected breast cancer events were calculated by applying age- and calendar period-stratified person-years of observation to corresponding Iowa Surveillance, Epidemiology, and End Results breast cancer incidence rates. Observed and expected events cumulated after accounting for death as a competing risk.

breast cancer, her lifetime risk doubles to 34%. Our data indicate that the Gail model predicts inaccurately for such women because the increased risk of breast cancer associated with atypia is independent of the effect of family history.

Women in our cohort with atypia and a positive family history of breast cancer had no additional increased risk of breast cancer over that of atypia alone. This finding counters the commonly held view proposed by the Nashville study (ie, that atypia and a positive family history increase breast cancer risk additively). When data from other major studies of benign breast disease are considered along with the Mayo findings, the preponderance of evidence calls into question the result from the Nashville group. In that study, the subgroup of women with atypia and a family history was small ( $n = 39$ ) with an RR of 8.9 (95% CI, 4.8 to 17), compared with 3.5 (95% CI, 2.3 to 5.5) in 193



**Fig 3.** Observed and expected cumulative breast cancer incidence among women with atypical hyperplasia, stratified by number of foci of atypia and histologic presence of calcifications. Expected events calculated by applying age- and calendar period-stratified person-years of observation among all women with atypia to corresponding Iowa Surveillance, Epidemiology, and End Results breast cancer incidence rates. Observed and expected events cumulated after accounting for death as a competing risk.

women with atypia and no family history.<sup>2</sup> In contrast, evaluation of a much larger population in the Breast Cancer Detection and Demonstration Project showed similar frequencies of breast cancer in women with atypia and family history (16 of 261, 6.1%) compared with those with atypia alone (51 of 1,044, 4.9%).<sup>4</sup> Recent data from the Nurses' Health Study confirm our finding that a family history of breast cancer in a first-degree relative does not further increase risk among women with atypical hyperplasia.<sup>15</sup> To explain these findings, we postulate that atypical hyperplasia is a phenotype reflecting increased risk; this phenotype derives from both inherited risk and lifetime exposures. Thus, the histologic presence of atypia already reflects the increased breast cancer risk inherent in a positive family history.

We have identified a new histologic variable that appears to stratify risk in women with atypia: multifocality. The RR of breast cancer increases in a dose-response fashion for women with one, two, and three or more foci of atypia, with a statistically significant test for trend. With a single focus, the cumulative incidence of breast cancer reached 18% at 25 years. For women with two or more foci of atypia, the cumulative risk of breast cancer was greater than 40% at 25 years. Moreover, in the highest risk subgroup of women with three or more foci and histologic calcifications, the cumulative incidence exceeded 50% over 25 years. This level of risk approaches that reported for carriers of BRCA mutations.<sup>16</sup> In line with our observation, differential risk based on extent of disease has been established for lobular neoplasia (ie, ALH v lobular carcinoma),<sup>17</sup> and the number of foci of atypia found in core needle biopsy specimens correlates with the likelihood of finding cancer at surgical excision.<sup>18</sup>

Some may question whether multifocal atypias may actually represent subtle in situ carcinoma, particularly those of the ADH type. In cases of multifocal ADH, it should be emphasized that individual foci arose in separate and distinct terminal duct lobular units, none of which measured more than 2 mm. Hence, these examples failed to exhibit the confluent degree of cellular proliferation requisite for a diagnosis of DCIS. We submit that more widespread distribution of atypical foci within breast tissue signals a larger burden of at-risk tissue that has progressed along the continuum toward breast cancer. The data presented in this article provide evidence that the extent of pre-malignant breast change is related to subsequent cancer risk. Since this is the first report of the clinical relevance of this histologic finding, we recognize the need for validation and plan to evaluate this factor in a more recent cohort from our institution. Furthermore, we hope that other research groups with large numbers of patients with atypical hyperplasia will also examine the relevance of multifocal atypia in their study sets.

Age at the diagnosis of atypia also emerged as a significant modifier of subsequent breast cancer risk, with a higher RR in younger women. The Nurses Health Study<sup>6</sup> and the Breast Cancer Detection and Demonstration Project<sup>8</sup> have also shown higher risk in younger women with atypia. In our cohort, this increased risk in younger women is not explained by more frequent multifocal disease or a positive family history. Perhaps atypical hyperplasia present at a young age is the result of previous oncogenic events; alternatively, breast tissue with atypia may be unusually susceptible to proposed oncogenic estrogen metabolites associated with the premenopausal hormonal environment.<sup>19</sup>

When counseling women with atypical hyperplasia, the length of time at risk is a key element in planning risk-reduction strategies. Dupont and Page<sup>9</sup> reported that the greatest risk of breast cancer after

a diagnosis of atypia lies in the first 10 years, with subsequent RR reduced by half ( $P = .06$ ). By contrast, the Nurses Health Study<sup>10</sup> found that risk does not decrease over time, with RR slightly higher more than 10 years after biopsy (RR, 3.6) compared with the first 10 years (RR, 3.2). Our data confirm that the RR for breast cancer after a biopsy demonstrating atypia remains significantly elevated for at least 15 years.

Data on long-term absolute risk are more useful than RR estimates when counseling patients. Our study provides estimates of absolute risk for women with atypia and indicates a higher cumulative incidence of breast cancer with long-term follow-up than has been reported by other studies. Figures from the study of Dupont and Page show a cumulative breast cancer incidence of 13% at 20 years and 23% at 25 years in women with atypia.<sup>9</sup> The cumulative incidences identified in our cohort were higher: 21% at 20 years and 29% at 25 years. One factor contributing to this difference is our inclusion of DCIS as a recordable breast cancer event, whereas the Nashville study counted only cases of invasive breast cancer.<sup>2</sup> Because DCIS currently receives local treatment (and in some cases, systemic treatment) similar to that for early-stage invasive breast cancer, it is reasonable to include cases of DCIS when estimating risk.

Our data on the laterality of subsequent breast cancer do not allow conclusions regarding atypical hyperplasia acting as a precursor lesion, yet there is a suggestion of predilection for the ipsilateral breast that requires ongoing study. Breast cancers occurring in the first 10 years after atypia diagnosis were significantly more likely to occur in the ipsilateral breast. A recent study of gene expression profiling identified remarkably similar alterations in gene expression among ADH, DCIS, and invasive cancers found in the same specimen, supporting the role of atypical hyperplasia as a precursor lesion.<sup>20</sup> Regarding differences in ipsilateral risk for ductal versus lobular atypia, we found that risk was equal for both breasts after a diagnosis of ALH, which is consistent with the distribution of invasive breast cancers after a diagnosis of lobular carcinoma in situ.<sup>21</sup> In contrast, ADH was more likely associated with a later ipsilateral breast cancer, as has been shown for DCIS untreated after diagnostic biopsy.<sup>22</sup>

In conclusion, our study provides a comprehensive analysis of breast cancer risk associated with atypical hyperplasia. These findings

confirm a four-fold RR of subsequent breast cancer in women with atypical hyperplasia. We estimate that the long-term absolute risk of subsequent breast cancer (in situ or invasive) is higher than previously reported—at least 25% at 25 years, and as high as 50% to 60% in a high-risk subgroup defined by multifocality and calcifications. A positive family history does not confer significantly increased risk in women with atypia. Improved risk prediction and stratification is now possible to guide risk-reduction counseling for women with atypical hyperplasia.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

#### AUTHOR CONTRIBUTIONS

**Conception and design:** Amy C. Degnim, Daniel W. Visscher, Marlene H. Frost, Thomas A. Sellers, Robert A. Vierkant, Karthik Ghosh, Thea Tlsty, L. Joseph Melton III, Lynn C. Hartmann

**Financial support:** Lynn C. Hartmann

**Administrative support:** Lynn C. Hartmann

**Provision of study materials or patients:** Wilma L. Lingle, L. Joseph Melton III, Lynn C. Hartmann

**Collection and assembly of data:** Amy C. Degnim, Daniel W. Visscher, Hal K. Berman, Marlene H. Frost, Thomas A. Sellers, Robert A. Vierkant, Shaun D. Maloney, Piet C. deGroen, Wilma L. Lingle, Lois Penheiter, Lynn C. Hartmann

**Data analysis and interpretation:** Amy C. Degnim, Daniel W. Visscher, Hal K. Berman, Marlene H. Frost, Thomas A. Sellers, Robert A. Vierkant, Shaun D. Maloney, V. Shane Pankratz, Karthik Ghosh, Carol A. Reynolds, Lynn C. Hartmann

**Manuscript writing:** Amy C. Degnim, Daniel W. Visscher, Thomas A. Sellers, Robert A. Vierkant, Shaun D. Maloney, V. Shane Pankratz, Piet C. deGroen, Karthik Ghosh, L. Joseph Melton III, Lynn C. Hartmann

**Final approval of manuscript:** Amy C. Degnim, Daniel W. Visscher, Hal K. Berman, Marlene H. Frost, Thomas A. Sellers, Robert A. Vierkant, Shaun D. Maloney, V. Shane Pankratz, Piet C. deGroen, Karthik Ghosh, Thea Tlsty, L. Joseph Melton, Carol A. Reynolds, Lynn C. Hartmann

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## Appendix B: Involution Paper

## ARTICLES

### Age-Related Lobular Involution and Risk of Breast Cancer

Tia R. Milanese, Lynn C. Hartmann, Thomas A. Sellers, Marlene H. Frost, Robert A. Vierkant, Shaun D. Maloney, V. Shane Pankratz, Amy C. Degnim, Celine M. Vachon, Carol A. Reynolds, Romaine A. Thompson, L. Joseph Melton III, Ellen L. Goode, Daniel W. Visscher

**Background:** As women age, the lobules in their breasts undergo involution or regression. We investigated whether lobular involution in women with benign breast disease was associated with subsequent breast cancer risk. **Methods:** We examined biopsy specimens of 8736 women in the Mayo Benign Breast Disease Cohort from whom biopsy samples were taken between January 1, 1967, and December 31, 1991. Median follow-up for breast cancer outcomes was 17 years. We classified lobular involution in the background breast tissue as none (0% involuted lobules), partial (1%–74%), or complete ( $\geq 75\%$ ). Subsequent breast cancer events and data on other risk factors were obtained from medical records and follow-up questionnaires. To estimate relative risks (RRs), standardized incidence ratios were calculated by use of incidence rates from the Iowa Surveillance, Epidemiology, and End Results (SEER) Registry. All statistical tests were two-sided. **Results:** Distribution of extent of involution was none among 1627 (18.6%) women, partial among 5197 (59.5%), and complete among 1912 (21.9%). Increased involution was positively associated with increased age and inversely associated with parity (both  $P < .001$ ). The relative risk for the entire cohort of 8736 women, compared with the Iowa SEER population, was 1.40 (95% CI = 1.30 to 1.51). Risk of breast cancer was associated with the extent of involution (for no involution, RR [i.e., observed versus expected] = 1.88, 95% confidence interval [CI] = 1.59 to 2.21; for partial involution, RR = 1.47, 95% CI = 1.33 to 1.61; and for complete involution, RR = 0.91, 95% CI = 0.75 to 1.10; test for heterogeneity  $P < .001$ ). Lobular involution modified risk in all subsets (e.g., among women with atypia, for no involution, RR = 7.79, 95% CI = 3.56 to 14.81; for partial involution, RR = 4.06, 95% CI = 3.03 to 5.33; and for complete involution, RR = 1.49, 95% CI = 0.41 to 3.82;  $P = .003$ ). **Conclusions:** In this large cohort of women with benign breast disease, lobular involution was associated with reduced risk of breast cancer. Aberrant involution may be a biologically important phenomenon in breast cancer biology. [J Natl Cancer Inst 2006;98:1600–7]

The mammary gland undergoes profound physiologic changes throughout the phases of a woman's life, including puberty, pregnancy, lactation, postlactational involution, and aging (1,2). The epithelial tissue of the human breast is organized into 15–20 major lobes, each made up of lobules that contain the milk-forming acini. As a woman ages, breast lobules regress, or involute, with a reduction in the number and size of

acini per lobule and replacement of the delicate intralobular stroma with the more dense collagen of breast connective tissue (Fig. 1) (1,3–5). Over time, there is progressive fatty replacement of glandular elements and collagen (1,5). This process differs greatly from postlactational involution. After lactation, there is regression of all breast tissues as secretory activity is curtailed, but there is no substantive loss of glandular tissue (1,2).

Although involution of the breast involves a consistent sequence of histologic changes, the rate and extent of involution vary considerably among individual women (3,4). This age-related lobular involution has been documented in women younger than age 40 years and thus involves factors not limited to the onset of menopause (1,3–5). Cowan et al. (4) studied age-related involution in breast tissue obtained at autopsy and speculated that obstetrical and lactational history may be more important than age at influencing the onset of involution, but they did not provide specific obstetrical data to support their claim. Geschickter (5) studied more than 100 breast specimens obtained at autopsy or surgery and noted the degree of involution by age and obstetrical history. He observed early changes consistent with lobular involution in 33% of women younger than age 40 years. He also found that repeated pregnancies were associated with the persistence of lobules, whereas lobule size and number declined in the absence of childbearing.

It has been hypothesized that age-related lobular involution, with its loss of glandular elements, may be associated with a decreased risk of breast cancer (6,7). To test this hypothesis, we characterized the extent of lobular involution by age group in a large cohort of women with benign breast disease and examined the association between involution and breast cancer risk.

**Affiliations of authors:** Mayo Medical School (TRM), Division of Medical Oncology (LCH, MHF, RAT), Division of Epidemiology (CMV, LJM, ELG), Division of Biostatistics (RAV, SDM, VSP), Division of General Surgery (ACD), and Division of Anatomic Pathology (CAR, DWV), Mayo Clinic, College of Medicine, Rochester, MN; Department of Cancer Prevention and Control, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL (TAS).

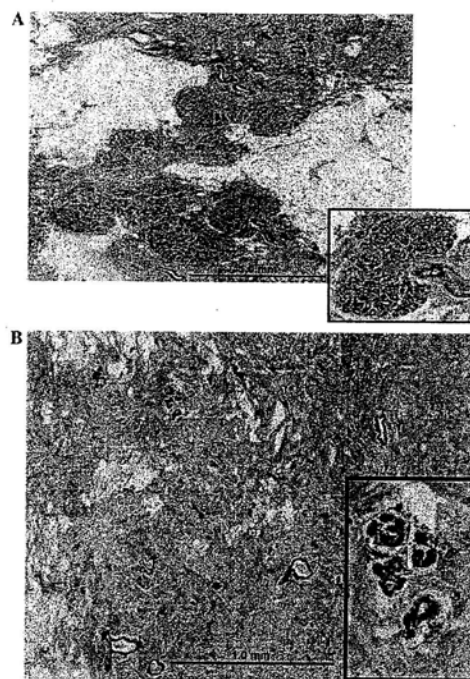
**Correspondence to:** Lynn C. Hartmann, MD, Department of Oncology, Mayo Clinic College of Medicine, 200 First St. SW, Rochester, MN 55905 (e-mail: hartmann.lynn@mayo.edu).

See "Notes" following "References."

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**Fig. 1.** Histologic features of age-related involution. A) Preinvolutional breast tissue with multiple intact terminal duct lobular units, each composed of multiple acini and specialized stroma (Inset). B) Complete lobular involution with mostly residual ducts with residual terminal duct lobular units, largely depleted of acini (Inset). Scale bars = 1.0 mm.

## PARTICIPANTS AND METHODS

### Study Population

Inclusion and exclusion criteria for the study cohort have been previously described (8). Briefly, the study population consisted of women aged 18–85 years who had benign breast disease (i.e., a breast biopsy examination with benign findings) diagnosed via surgical excision at Mayo Clinic between January 1, 1967, and December 31, 1991. The Mayo Benign Breast Disease Cohort included 9087 women with 15 years of follow-up at initial report (8). Since that report, we obtained cancer follow-up data for an additional 289 women, for whom that information was lacking previously, bringing the cohort to a total of 9376 women with a median of 17 years of follow-up. For 640 breast biopsy samples, the biopsy specimen consisted entirely of the index lesion; there was no background breast tissue in which to determine the degree of lobular involution. Thus, the final cohort for this analysis included 8736 women.

All protocol procedures and patient contact materials were reviewed and approved by the Institutional Review Board of the Mayo Clinic. Return of the patient contact materials was considered implied consent.

### Histology

All slides were reviewed by a breast pathologist (DWV) without knowledge of patient age, cancer outcome, or original histologic diagnosis. Biopsy findings were classified by the most extreme degree of hyperplasia as nonproliferative, proliferative disease without atypia, or atypical hyperplasia, as previously reported (8).

Each biopsy specimen was also categorized according to the extent of lobular involution in the background breast tissue. Involutional terminal duct lobular units (TDLUs) contain only a few to several small acini that may be distended by cystic change (Fig. 1). Involutional lobules also have flattened inconspicuous acinar epithelium with fibrosis of specialized intralobular stroma. The degree of involution for each specimen was categorized as none (0% TDLUs involuted), partial (1%–74% TDLUs involuted), or complete ( $\geq 75\%$  TDLUs involuted). These cut points were set by the pathologists at the initiation of the study to best distinguish the extremes of no involution from near-complete involution.

In general, viewing five to six lobules was sufficient to assess the extent of involution. One slide from a breast specimen typically contained a dozen or more lobules. There are two exceptions to this statement: 1) when involution was extensive and there are only a few lobular remnants on the slide (which is sufficient to state that complete lobular involution has occurred) and 2) when the entire sample consists of an epithelial hyperplastic lesion, as was the case for 640 (6.8%) of the 9376 women in our original cohort.

### Risk Factor Information and Follow-up

To obtain information about family history, reproductive history, and use of hormone replacement therapy, a study-specific questionnaire was sent to patients; 5352 (61%) of the 8736 women or their next of kin returned the questionnaire. Follow-up for breast cancer events was obtained through comprehensive (inpatient and outpatient) Mayo medical records and the questionnaire.

Family history of breast cancer was categorized as strong, weak, or negative. A strong family history was defined as the patient having 1) at least one first-degree relative with breast cancer diagnosed before age 50 years or 2) two relatives with breast cancer at any age, with at least one being a first-degree relative. Patients with family history of breast cancer who did not meet the above criteria were categorized as having a weak family history (8).

### Statistical Analysis

Data were summarized descriptively by use of frequencies and percentages. We initially compared the unadjusted distribution of breast cancer risk factors across levels of involution with chi-square tests of statistical significance. Subsequent comparisons were made after accounting for the effects of age by use of multivariable, nominal logistic regression analysis (9). We summarized results from these analyses by use of adjusted percentages, carried out by calculating log odds estimates for each 10-year age category (<40, 40–49, 50–59, 60–69, 70–79, or  $\geq 80$  years), back-transforming to percent estimates, and then averaging the corresponding percentages across all sets of age. This approach was similar to a least-squares means estimate in an analysis of variance setting. Among 245 women with synchronous bilateral biopsy examinations, we assessed the level of agreement across the two readings by use of weighted kappa statistics and their corresponding 95% confidence intervals (CIs).

The length of follow-up for each woman in the study was calculated as the number of days from her biopsy examination to the date of her breast cancer diagnosis, death, or last contact. We estimated relative risks (RRs) on the basis of standardized incidence ratios by dividing the observed numbers of incident breast cancers by expected numbers of population-based incident breast cancers. Expected values were calculated by apportioning each woman's person-years of follow-up into 5-year age and calendar-period categories and multiplying these by the corresponding breast cancer incidence rates from the Iowa Surveillance, Epidemiology, and End Results (SEER) Registry. This reference population was chosen because of its demographic similarities to the Mayo Clinic population (80% of cohort members reside in the upper Midwest). Potential heterogeneity in relative risks across levels of involution was assessed by use of Poisson regression analysis, with the log-transformed expected event rate for each individual modeled as the offset term.

In addition to assessing overall breast cancer risk, we also compared rates of ipsilateral to contralateral breast cancer in relation to the side of the benign lesion, both overall and by levels of involution. When calculating incidence for ipsilateral cancer, individuals with contralateral cancer were censored at their date of diagnosis, and vice versa. Women with missing laterality information, bilateral biopsy examination results, or bilateral breast cancer were censored for both events in these analyses. This approach yielded identical numbers of person-years for each type of event. As a result, the length of follow-up was not a factor in the analysis, and the rate comparisons reduced to simple comparisons of the number of events. Thus, we were able to assess whether the relative rate of ipsilateral cancer (compared with contralateral cancer) differed across levels of involution using simple chi-square tests of statistical significance. All statistical tests were two-sided, and all analyses were carried out with the SAS software system (SAS Institute, Inc, Cary, NC).

## RESULTS

### Extent of Lobular Involution

We characterized the extent of lobular involution in the benign breast biopsies of a cohort of 8736 women with tissue sampled between January 1, 1967, and December 31, 1991, at the Mayo Clinic. The distribution of the patients by level of lobular involution was as follows: no involution among 1627 (18.6%) women, partial involution among 5197 (59.5%) women, and complete involution among 1912 (21.9%) women.

### Factors Associated With Involution

As shown in Table 1, the degree of lobular involution increased progressively with age at diagnosis of benign breast disease ( $P < .001$ ). Complete involution of lobular units was observed in only 19 (3.4%) of the 566 women who were younger than 30 years at their benign biopsy; in 53 (5.1%) of the 1037 women aged 30–39 years; in 142 (5.8%) of the 2446 women aged 40–49 years; in 455 (21.6%) of the 2109 women aged 50–59 years; in 724 (45.3%) of the 1600 women aged 60–69 years; and in 519 (53.1%) of the 978 women aged 70 years or older. The gradual nature of the involution process is apparent in that it is already present at least to a partial degree in more than half of the women younger than 40 years and is still ongoing in women older than 70 years.

**Table 1.** Association of lobular involution with age at diagnosis of benign breast disease, family history of breast cancer, parity, lactation, and hormone replacement therapy\*

Characteristic	Extent of lobular involution, No. (%)			P value†
	None	Partial	Complete	
Overall	1627 (18.6)	5197 (59.5)	1912 (21.9)	
Age at BBD, y				<.001
18–29	308 (54.4)	239 (42.2)	19 (3.4)	
30–39	417 (40.2)	567 (54.7)	53 (5.1)	
40–49	643 (26.3)	1661 (67.9)	142 (5.8)	
50–59	218 (10.3)	1436 (68.1)	455 (21.6)	
60–69	29 (1.8)	847 (52.9)	724 (45.3)	
≥70	12 (1.2)	447 (45.7)	519 (53.1)	
Family history of breast cancer‡				<.001
None or weak	796 (21.1)	2717 (56.4)	911 (22.4)	
Strong	223 (24.5)	566 (55.9)	139 (19.5)	
Parity§				<.001
Nulliparous	113 (17.6)	421 (55.3)	177 (27.1)	
Parous	893 (22.6)	2780 (56.2)	836 (21.2)	
No. of children¶				<.001
0	113 (17.6)	421 (55.3)	177 (27.1)	
1	75 (17.2)	269 (54.7)	131 (28.0)	
2	324 (22.2)	891 (56.2)	258 (21.5)	
3	257 (23.9)	742 (55.8)	205 (20.4)	
≥4	235 (26.6)	871 (55.3)	242 (18.0)	
Children breastfed				.428
No	464 (21.6)	1555 (57.4)	436 (21.1)	
Yes	431 (23.3)	1202 (55.9)	364 (20.8)	
Hormone replacement therapy¶¶				.016
Ever	481 (22.5)	1330 (57.1)	494 (20.3)	
Never	516 (21.8)	1837 (55.4)	458 (22.9)	

\*For age at diagnosis of benign breast disease (BBD), percentage values were unadjusted. For all other variables, percentage values were adjusted for age.

†For age at BBD,  $P$  values were calculated using chi-square tests of statistical significance. For all other variables,  $P$  values were calculated by use of multivariate logistic regression analyses, accounting for the effects of age. All statistical tests were two-sided.

‡Information on family history was available for 5352 of 8736 women.

§Information on parity was available for 5220 of 8736 women. Specific number of children was available for 4500 of the 4509 parous women.

||Information on breastfeeding was available for 4452 of 8736 women.

¶¶Information on hormone replacement therapy was available for 5116 of 8736 women.

We also found a strong, inverse association ( $P < .001$ ) between lobular involution and parity (Table 1). Specifically, the likelihood of complete involution was 27.1% (95% CI = 24.1% to 30.1%) in nulliparous women, 28.0% (95% CI = 24.7% to 31.4%) in women who had one child, 21.5% (95% CI = 19.3% to 23.8%) in women who had two children, 20.4% (95% CI = 17.8% to 23.0%) in women who had three children, and 18.0% (95% CI = 16.1% to 20.0%) in women who had four children or more.

Separating women into categories of ever versus never breastfeeding did not reveal any relationship with extent of lobular involution ( $P = .428$ ). Women who reported having used hormone replacement therapy were slightly less likely to have complete involution (20.3%) than those with no history of hormone replacement therapy use (22.9%) ( $P = .016$ ). Breast tissue from women with a strong family history of breast cancer was less likely than that from women with no or a weak family history of breast cancer to demonstrate lobular involution; i.e., after adjustment for age, more women with a strong family history had no involution (24.5%) than those with no or a weak family history

Table 2. Association of involution and other risk factors with breast cancer after the diagnosis of benign breast disease

Characteristic	No. of women	No. of person-years	No. of observed events	No. of expected events*	Relative risk (95% CI)†
Degree of involution					
None (0%)	1627	32 271	150	79.6	1.88 (1.59 to 2.21)
Partial (1%-74%)	5197	90 409	440	300.1	1.47 (1.33 to 1.61)
Complete (≥75%)	1912	28 376	106	116.5	0.91 (0.75 to 1.10)
Histologic type					
Nonproliferative	5736	101 201	355	321.5	1.10 (0.99 to 1.23)
Proliferative without atypia	2677	45 418	276	158.1	1.75 (1.55 to 1.96)
Proliferative with atypia	323	4436	65	16.6	3.91 (3.02 to 4.98)
Age at biopsy, y					
<45	2682	52 055	158	108.4	1.46 (1.24 to 1.70)
45-55	2559	49 246	254	169.0	1.50 (1.32 to 1.70)
≥55	3495	49 754	284	218.8	1.30 (1.15 to 1.46)
Family history of breast cancer‡					
None or weak	4424	81 514	329	269.4	1.22 (1.09 to 1.36)
Strong	928	18 385	115	59.5	1.93 (1.59 to 2.32)
Age at birth of first live child, y§					
Nulliparous	711	13 021	71	41.1	1.73 (1.35 to 2.18)
<30	4121	77 710	327	257.9	1.27 (1.13 to 1.41)
≥30	388	7091	30	22.4	1.34 (0.90 to 1.92)

\*Number of events expected on the basis of Iowa Surveillance, Epidemiology, and End Results breast cancer incidence data.

†All analyses account for the effects of age and calendar period. CI = confidence interval.

‡Information on family history of breast cancer was available for 5352 of 8736 women.

§Information on parity was available for 5220 of 8736 women.

(21.1%), and fewer women with a strong family history had complete involution (19.5%) than those with no or a weak family history (22.4%) (logistic regression analysis comparing distribution of involution across levels of family history,  $P < .001$ ).

We also examined the extent of lobular involution by category of benign breast disease. Among women with nonproliferative disease, 27.2% had complete involution. However, among women with proliferative disease without or with atypia, only 11.5% had complete lobular involution ( $P < .001$ ).

#### Lobular Involution and Breast Cancer Risk

This cohort of women with benign breast disease was, overall, at increased risk of breast cancer when compared with age-matched women in the general population. Specifically, the relative risk for the entire cohort of 8736 women, compared with the Iowa SEER population, was 1.40 (95% CI = 1.30 to 1.51). In our cohort, degree of involution was associated inversely with breast cancer risk (Table 2; e.g., for no involution, RR = 1.88, 95% CI = 1.59 to 2.21; for partial involution, RR = 1.47, 95% CI = 1.33 to 1.61; and for complete involution, RR = 0.91, 95% CI = 0.75 to 1.10; test for heterogeneity  $P < .001$ ).

Figure 2 illustrates the observed associations between the extent of involution and breast cancer risk among strata of age, histology, family history, and parity. Extent of lobular involution modified age-related breast cancer risk (e.g., for a woman older than 55 years with no involution, RR = 3.21, 95% CI = 1.90 to 5.08, and for a similar woman with complete lobular involution, RR = 0.92, 95% CI = 0.74 to 1.14). The same pattern was observed in all age groups.

Similarly, progressive increases in lobular involution in background breast tissue was associated with reduced risk of breast cancer among women with benign proliferative disease, even those with atypia (Fig. 2). Among women with atypia, no involution was more strongly associated with a higher risk of breast cancer (RR = 7.79, 95% CI = 3.56 to 14.81) than complete involution (RR = 1.49, 95% CI = 0.41 to 3.82) or partial involution (RR =

4.06, 95% CI = 3.03 to 5.33) (test for heterogeneity,  $P = .003$ ). Among women with proliferative disease without atypia, no involution was also associated with a higher risk of breast cancer (RR = 2.94, 95% CI = 2.26 to 3.75) than complete involution (RR = 1.11, 95% CI = 0.68 to 1.71). The same pattern held true for those with nonproliferative breast disease; i.e., those with no involution had a higher risk than those with complete or partial involution.

Lobular involution modified the influence of family history on risk of breast cancer (Fig. 2). Among women with a strong family history of breast cancer, no involution was associated with the highest risk of breast cancer (RR = 2.77, 95% CI = 1.94 to 3.84), followed by partial involution (RR = 1.72, 95% CI = 1.32 to 2.20) and then by complete involution (RR = 1.61, 95% CI = 0.92 to 2.61). Among women with no or a weak family history of breast cancer and complete involution, the risk of breast cancer (RR = 0.59, 95% CI = 0.41 to 0.81) was approximately half of that for the general population, which was based on Iowa SEER data, and approximately fivefold less than the risk of those with strong family history and no involution (i.e., RR = 2.77, 95% CI = 1.94 to 3.84).

Lobular involution also modified the risk associated with parity or age at birth of the first live child. Among nulliparous women and women whose first live child was born when she was at least 30 years old, no lobular involution was associated with increased risks of breast cancer (RR = 2.41, 95% CI = 1.25 to 4.21, and RR = 2.74, 95% CI = 1.31 to 5.03, respectively). However, among these same two groups of women, when complete lobular involution had occurred, there was no increase in risk (RR = 1.02, 95% CI = 0.53 to 1.78, and RR = 0.48, 95% CI = 0.10 to 1.40, respectively). Among women whose first live child was born when she was younger than 30 years old, complete lobular involution was associated with a decreased risk of breast cancer (RR = 0.65, 95% CI = 0.44 to 0.91).

We also asked whether the era in which the biopsy examination was performed—namely, before or after widespread adoption of mammography—affected the results. In the first 15 years of the cohort (from 1967 through 1981), 78% of biopsy examinations were done because of a palpable concern (i.e., a palpable

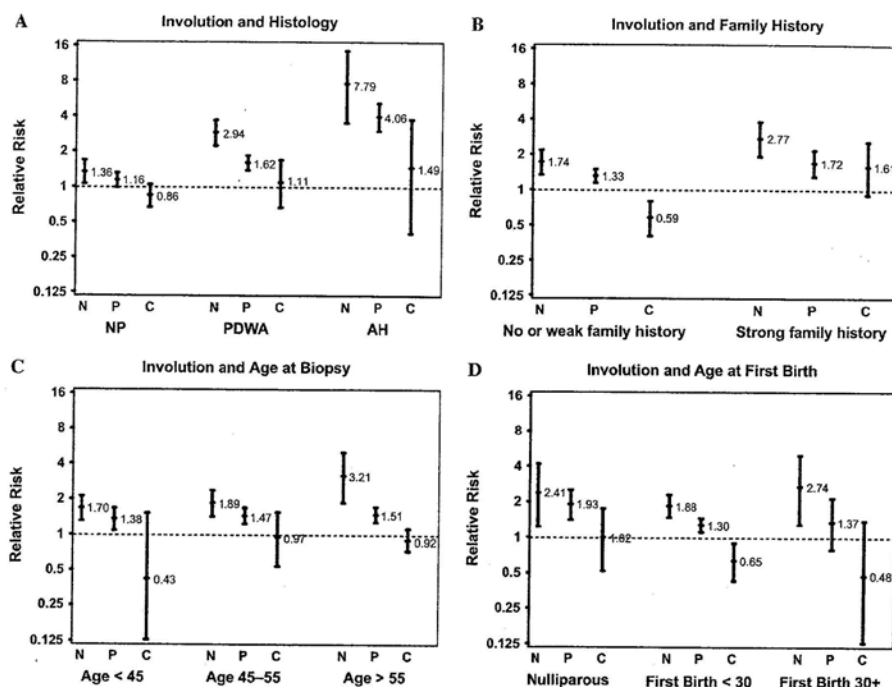


Fig. 2. Association of breast cancer risk with lobular involution and other variables. Relative risks (as indicated) and their 95% confidence intervals (error bars) reflect the observed number of events compared with the number of expected events on the basis of Iowa Surveillance, Epidemiology, and End Results data. All results account for the effects of age and calendar period.

A) Involution and histology. B) Involution and family history. C) Involution and age at biopsy examination. D) Involution and age at birth of first live child. N = no involution; P = partial involution; C = complete involution; NP = nonproliferative; PDWA = proliferative disease without atypia; AH = atypical hyperplasia.

lump detected during a clinical breast examination or by the patient), and 22% were done because of an abnormal mammogram. From 1982 through 1991, 40% of the biopsy examinations were done because of a palpable concern, and 60% were done because of an abnormal mammogram. The relative risks of breast cancer by involution status and by dates (Table 3) indicated that associations between extent of involution and risk were similar in the pre- and postmammography time periods.

#### Lobular Involution: Localized or Field Effect

To address whether or not the degree of involution was relevant only to the area of the biopsy or was representative of the field of breast tissue, we examined 1) whether, for women with bilateral benign biopsy examination results, involution results were concordant and 2) whether the degree of involution at the benign biopsy site was associated with the risk of ipsilateral breast cancer or with both ipsi- and contralateral breast cancers. A subset of 245 women had bilateral biopsy examinations performed at the same time. In 203 (83%) of these women, the same category of involution (no, partial, or complete) was found in the biopsy tissue from both breasts. In 41 (17%), there was a difference of one category between the two breasts. Only one individual had complete involution in the biopsy tissue of one breast and no involution in the

contralateral sample. These results indicate a high level of agreement in involution measured across multiple biopsy specimens within a woman (kappa coefficient = 0.72, 95% CI = 0.64 to 0.80; test for agreement beyond that expected by chance  $P < 0.001$ ).

We next investigated the extent of involution and the laterality of subsequent breast cancers. In our cohort overall, there is a slight predominance of ipsilateral breast cancers (55.5%) over contralateral breast cancers (44.5%), as reported previously (8); this result is thought to reflect the presence of some direct precursors among these lesions. To determine whether involution at the site of the benign breast disease was relevant to the contralateral breast, we examined the ratio of ipsilateral to contralateral events by degree of involution. With no involution, the ratio was 53.6% ipsilateral to 46.4% contralateral; for partial involution, the ratio was 55.9% to 44.1%; and for complete involution, the ratio was 53.5% to 46.5% (chi-square test for difference in percent ipsilateral across involution status,  $P = .85$ ). Thus, the relationship between involution extent and breast cancer risk was observed in both the ipsi- and contralateral breast.

#### DISCUSSION

We characterized the degree of lobular involution in the background breast tissue in a large cohort of women with benign

Table 3. Relative risk of breast cancer by involution and year of biopsy

Extent of involution and year of biopsy	No. of women	No. of person-years	No. of observed events	No. of expected events*	Relative risk (95% CI)†
No involution					
1967-1981	957	21 886	105	56.9	1.85 (1.51 to 2.23)
1982-1991	670	10 384	45	22.7	1.98 (1.44 to 2.65)
Partial involution					
1967-1981	2381	49 080	204	157.3	1.53 (1.34 to 1.73)
1982-1991	2816	41 329	200	142.8	1.40 (1.21 to 1.61)
Complete involution					
1967-1981	740	12 524	55	46.8	1.18 (0.89 to 1.53)
1982-1991	1172	15 851	51	69.7	0.73 (0.54 to 0.96)

\*Number of events expected on the basis of Iowa Surveillance, Epidemiology, and End Results breast cancer incidence data.

†All analyses account for the effects of age and calendar period. CI = confidence interval.

breast disease. Our data demonstrate a strong, inverse relationship between degree of involution and breast cancer risk. To our knowledge, this is the first study to systematically examine age-related involution in the context of breast cancer risk. Furthermore, greater degrees of involution reduced breast cancer risk even in high-risk subsets defined by age, atypia, reproductive history, or family history. There was a strong direct association between involution and increasing age. There was an inverse association between involution and parity.

As in this study, others have found that older women tend to have fewer lobules or only lobule remnants (4,5). Cowan and Herbert (4) performed a detailed autopsy study of the breast tissue from 102 women, aged 50-104 years, who died without known breast disease. Although considerable individual variability was present, they described a progressive loss of lobules with increasing age. Earlier reports state that age-related involution has already begun in women under the age of 40 years (1,5). Our data confirm that this process is present, at least to a partial degree, in many younger women.

We hypothesize that the degree of involution detected at the benign biopsy site reflects that of the overall field of a woman's breast tissue. We believe that this hypothesis is reasonable because of our results showing a similar likelihood of contralateral and ipsilateral breast cancers by involution status at the site of the benign breast disease and because of the high concordance in involution status in women who had bilateral biopsy examinations. However, our study design cannot answer this question definitively. To do so would require examination of the extent of involution throughout all of a woman's breast tissue.

It is widely appreciated that, as women age, their risk of breast cancer increases. But the rate of increase of breast cancer slows appreciably at approximately age 50 years (10,11), which has been attributed to a reduction in ovarian hormonal production (12). We observed a definite increase in the process of involution at approximately age 50 years (with complete involution present in 5.8% of women aged 40-49 years and in 21.6% of women aged 50-59 years). These data raise the possibility that involution may be contributing to the slowing in the rate of increase of breast cancer among women older than 50 years, as speculated by Henson and Tarone (7).

We examined various factors besides age for their association with degree of involution. We found an inverse association between lobular involution and parity. Others have also reported that the more children a woman has, the more likely she is to have persistence of lobular structures (1,5), which we found was associated with increased risk of breast cancer. Yet, multiparity is

generally considered to reduce the risk of breast cancer (13,14). Several factors may explain this apparent contradiction. First, we do not have data on the age at each child's birth for the women in our cohort. Some epidemiologic work has suggested that full-term pregnancies after 35 years of age are associated with an increased risk of breast cancer (7,15). Thus, data on a woman's age at each pregnancy and on her age at breast biopsy examination would help to evaluate more definitively the relationships of parity, involution, and breast cancer risk. In addition, our study was limited by the relatively large size of the group of women categorized as having partial involution. More specific, quantitative measures of degree of involution should be explored to determine whether the association between parity status and degree of involution can be defined more precisely. Given the inverse association between complete involution and multiparity and given that both are associated with reduced risk of breast cancer, we hypothesize that the breast cancer risk modification associated with parity is independent of involution status.

There are several biologic mechanisms by which involution or lack thereof could alter a patient's breast cancer risk. The decrement in epithelial cell number that accompanies involution may decrease breast cancer risk simply because there are fewer epithelial cells to undergo malignant transformation. Another possibility is that aberrant involution may be a marker or phenotype reflecting underlying constitutional susceptibility for breast cancer that is present in the epithelial or stromal compartment or in their relationship with each other. Yet another possibility is that failure to undergo timely or appropriate involution allows prolonged exposure of epithelial cells to intrinsic and/or extrinsic mutagenic stresses (16-20). In this model, the prime targets of such mutagenic processes, such as stem cells or early progenitors, may become quiescent during the process of involution. Experiments to characterize the epithelial and stromal mediators present in tissue with and without involution, in women with and without subsequent cancer, should help to clarify the mechanism of risk reduction.

For our work to date, we divided extent of involution into three categories. We recognized that, although the morphologic patterns of age-related lobular involution have been defined (1,4,5), no histologic standard exists for evaluating the extent of breast involution. In particular, there is no well-characterized method for grading partial degrees of involution. For this reason, we attempted to classify degree of involution with the least amount of subjectivity. Thus, by deciding only whether breast tissue had no lobular involution versus almost complete involution and then by combining the remainder into one category of partial involution, we minimized the subjectivity inherent in judging percent involution.

Our study has several limitations. First, these findings do not necessarily pertain to all women because the cohort studied included women who had a breast biopsy because of some concern. Nevertheless, women with benign breast disease make up a large population who are understandably concerned about their breast cancer risk (estimated at 1 million US women each year) (21–23). Another limitation lies in our current very broad category of partial involution. This category encompasses a wide range of involution extent (1%–74% of lobules involuted). We expect that more specific gradations would support more refined association studies. Finally, we did not have complete risk factor data for all the women in the cohort, largely because the women with biopsy examinations in the earlier years of the cohort are now elderly or deceased. Fortunately, for purposes of this report, we did not have to depend on the questionnaire for involution status or for cancer outcomes (which were available from our comprehensive Mayo medical record). We had completed questionnaires for 63.8% of the patients with breast cancer and 61.6% of the patients without breast cancer in the cohort.

There are other approaches to the study of involution and breast cancer risk. Henson and Tarone (7) suggested an autopsy case-control series to look at involution as a possible risk factor for breast cancer. Although this approach would provide access to extensive amounts of breast tissue, the availability of clinical risk factor information and of a sufficient number of subjects could be limiting. Other women who have breast tissue removed in the course of clinical care are those who have reduction mammoplasty or prophylactic mastectomy. These women are, respectively, those who have breast hypertrophy or a hereditary predisposition to breast cancer. Although involution (or lack thereof) in these women is of considerable interest, their tissue is not necessarily representative of the general population.

The mechanisms controlling age-related involution are of considerable interest. Molecular programs that control postlactational involution in rodents have been studied extensively (24). With postlactational involution, there is dramatic reversal of the developmental changes wrought by pregnancy. Specifically, there is widespread apoptosis of alveolar epithelial cells followed by removal of apoptotic debris and remodeling of the stroma and extracellular matrix (24). These events occur within a matter of days of abrupt weaning and restore the gland to its pre-pregnancy state. In contrast, the molecular orchestration of age-related involution, to our knowledge, has not yet been characterized.

In the past, for women with benign breast biopsy results, the type and extent of epithelial proliferation present in their biopsy has been the principal way to stratify their risk. Results of our study indicate that assessing the status of lobular involution in the biopsied tissue may ultimately add to risk prediction capabilities. It is notable, as shown in Fig. 2, C, that some of the most extreme risk estimates are observed in women whose involution status is unusual for their age—namely, young women with complete involution (RR = 0.43, 95% CI = 0.13 to 1.55) and women older than 55 years with no involution (RR = 3.2, 95% CI = 1.90 to 5.08). It is tempting to speculate that the process of complete involution may be protective and, conversely, that lack of involution identifies higher risk groups. However, confidence intervals were wide around the estimates for these less common categories.

In summary, we have evaluated the extent and effect of age-related lobular involution in a cohort of approximately 9000

women who had a benign breast biopsy examination. We observed a statistically significant reduction in risk of breast cancer among those women whose breast tissue had undergone extensive lobular involution, which was apparently independent of other markers of risk. Among women with benign breast disease, assessment of extent of involution may help to fine-tune current risk prediction approaches. Elucidation of the mechanism of lobular involution may reveal ways to promote the process as a means of risk reduction.

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#### NOTES

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## Appendix C: Editorial Accompanying Involution Paper

# EDITORIALS

## Lobular Involution: the Physiological Prevention of Breast Cancer

*Donald Earl Henson, Robert E. Tarone, Hala Nsouli*

It truly is a remarkable event when traditional pathologic observations lead to new ideas about the prevention of cancer. In this issue of the Journal, Milanese et al. (1), through a histologic review of breast biopsy specimens, show that the extent of age-related lobular involution is strongly associated with a reduced risk of breast cancer. Breast cancer risk decreased with increasing extent of involution in both high-risk and low-risk subgroups defined by age, epithelial atypia, reproductive history, and family history of breast cancer.

Beginning in the premenopausal period, lobular involution is a physiologic process that occurs over many years whereby the parenchymal elements in the breast progressively atrophy and disappear (2, 3). The study reported by Milanese et al. represents a unique application of the Mayo Benign Breast Disease Cohort to investigate prospectively involution as a risk factor for breast cancer. It is the first study, to our knowledge, to substantiate a hypothesis that is based on pathologic and epidemiologic considerations that delayed involution is a major risk factor for breast cancer (4, 5).

As for an explanation of the effect of lobular involution on breast cancer risk, it has been suggested that a reduction in mammary gland tissue that results from involution should lead to a reduction in breast cancer because a progressively smaller amount of epithelial tissue is available for malignant transformation (1, 5). The result of involution, therefore, can be considered physiologically analogous to a partial prophylactic mastectomy, with a corresponding reduction in breast cancer risk.

Although a reduction in mammary tissue is a plausible explanation, the underlying issue is one of aging or, more precisely, the failure of breast tissue to age normally. The aging process in the breast is under control of various hormones and does not follow the pattern seen in other organs or tissues. Pathologists have long commented on the possibility that persistent atypical lobules might be precursors of invasive breast cancer (6, 7). It seems paradoxical that an organ that normally undergoes complete or near complete physiologic atrophy would be a site in which cancer rates steadily increase with age. The continuing increase in breast cancer risk with age is likely associated with the persistence of glandular epithelium beyond the time of normal involution, reflecting an abnormal delay of the aging process in the breast (4, 5).

Except for morphologic observations concerning age of onset and progression with age, practically nothing is known about the process of involution. Even less is known about factors that

control involution or that delay or accelerate the process. In this context, it is unknown whether the rate of involution is genetically determined and whether known breast cancer risk or environmental factors alter the rate of involution.

Evidence indicates that some risk factors for breast cancer may interfere or affect the process of involution. In the Mayo study, women with benign proliferative breast disease were substantially less likely to have complete involution than were women with benign nonproliferative disease, and women with a strong family history of breast cancer had slightly less advanced involution than women without such history (1). Late age of menopause, which increases the risk of breast cancer, is likely to result in delayed involution because of persistence of estrogen activity (8). Women whose first full-term pregnancy occurs after age 35 years have an elevated risk for breast cancer compared with nulliparous women or with women whose first pregnancy was at a much younger age (9, 10). After the commencement of involution, late pregnancy with its concomitant increase in the proliferation of the ductal-alveolar epithelium is likely to interrupt the normal process of involution, which typically begins between 30 and 40 years of age. Oophorectomy, which protects against breast cancer (11), leads to the same type of atrophy of breast parenchyma in young women as that seen in older women (12). The reduction in risk may be due to the acceleration of involution induced by oophorectomy.

One of the most striking findings in the study of Milanese et al., however, is the degree to which the strong association between extent of involution and breast cancer risk was independent of all known breast cancer risk factors that were investigated (1). This observation suggests that factors unrelated to known risk factors are responsible for the protective effect of involution. For this reason, a greater understanding of the biologic basis for involution will be required to elucidate the mechanisms of the protective effect of lobular involution on breast cancer risk.

The observations reported by the Mayo group may find practical applications for risk prediction (1). It may be useful for pathologists to report the extent of involution in addition to any epithelial changes found in breast biopsy specimens that do not contain cancer. It will be important to determine the extent to which mammographic breast density serves as a surrogate for the extent of involution. By taking extent of involution into account, it should be possible to increase the predictive ability of breast cancer risk models.

Results of the Mayo study provide a new paradigm

for breast cancer research and prevention. Age has always seemed the opponent because of the increasing risk of breast cancer with age, but age may now become an ally. The challenge will be to unravel the natural history of involution and the normal process of aging in the breast. Eventually, involution could become a useful surrogate endpoint for research in breast cancer prevention. A possible approach to prevention may be to develop strategies that achieve complete involution as early as possible after childbearing is completed.

*Affiliations of authors:* Office of Cancer Prevention and Control (DEH) and Department of Epidemiology and Biostatistics, School of Public Health and Health Services (HN), The George Washington University Cancer Institute, Washington, DC; International Epidemiology Institute, Rockville, MD (RET); Department of Medicine and Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN (RET).

*Correspondence to:* Donald E. Henson, MD, Office of Cancer Prevention and Control, The George Washington University Cancer Institute, Ross Hall, Rm. 502, 2300 "I" St. NW, Washington, DC 20037 (e-mail: patdeh@ gwumc.edu ).

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## NOTE

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